762-Pos Board B531
A Single-Molecule Study to Resolve How Kinases Prevent Chromosomal Mis-Segregation
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Mitosis is an exquisitely choreographed process that relies on specialized interactions between kinetochores on chromosomes and the microtubules of the mitotic spindle. The process is orchestrated by an error-correction system that, remarkably, detects improperly aligned chromosomes by sensing a lack of tension in their kinetochore-microtubule attachments. This surveillance system ensures that each daughter cell receives exactly one copy of each chromosome; failure leads to cancer and birth defects. Two surveillance system components, the Mps1 and Ipl1 protein kinases, are of particular interest because indirect evidence suggests that they are responsible for core system functions. Direct tests of their functions have been lacking because the tension that they are thought to sense cannot be accurately measured or controlled in a cell. Very recently, our group completed the first-ever study of kinetochore-microtubule attachments reconstituted in vitro, positioning me to study the tension-sensing surveillance system in ways never before possible. I will apply precise forces to kinetochore-microtubule attachments using an optical trap, and I will make genetic changes to the kinetochores at sites where the kinases are thought to act. With these powerful single-molecule techniques, I will uncover how Mps1 and Ipl1 (1) modify kinetochores to destabilize their microtubule attachments, and (2) operate on unattached, improperly attached, and properly attached kinetochores to promote the formation of proper attachments. Ultimately, my work will guide efforts currently underway to develop new chemotherapeutic inhibitors of Ipl1 and Mps1. It will also help us to understand how cells sense and respond to force at the molecular level in other contexts.

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Insights into the Micromechanical Properties of the Metaphase Spindle
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During cell division, the microtubule-based metaphase spindle is subjected to mechanical forces that act in diverse orientations and over a wide-range of timescales. Currently, we cannot explain how this micron-sized, dynamic cytoskeletal structure generates and responds to forces while maintaining overall stability, as we have a poor understanding of its micromechanical properties. Here we combine the use of force-calibrated needles, high-resolution microscopy, and biochemical perturbations to analyze the vertebrate metaphase spindle's timescale- and orientation-dependent viscoelastic properties. We find that the metaphase spindle is mechanically anisotropic, and deforms either elastically or viscously depending on the timescale of applied force. We also find that spindle viscosity depends on the dynamics of microtubule crosslinking and the density of the filament. Spindle elasticity can be linked to the rigidity of kinetochore and non-kinetochore microtubules, which have different polymerization dynamics and stability, and also to spindle pole organization by kinesin-5 and dynein. These data suggest a quantitative model for the micromechanics of this cytoskeletal architecture and provide insight into how structural and functional stability is maintained in the face of different forces, such as those that control spindle size and position, and can result from deformations associated with chromosome movement.

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Symmetrical Shape of the Meiotic Spindle is Dynamically Balanced
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Meiotic spindle assembly is critical for achieving accurate chromosome segregation. The spindle mainly consists of microtubules and molecular motors. Recent studies have suggested that bipolar spindle formation requires the force balance sustained by molecular motors and polymerization-depolymerization dynamics of microtubules. However, it is not well understood whether and how the two pole structures are symmetrically balanced in a spindle. In this study, we quantitatively measured the mechanical stiffness, the microtubule density, and the response to the deformation of spindle poles by micromanipulation techniques and 3D analysis. To deform the spindle self-assembled in *Xenopus* egg extract, we inserted two glass micro-needles into a pole region at one side and widened it perpendicularly to the pole-to-pole axis. We found that the stiffness and the microtubule density in the manipulated side of pole region reduced upon widening. Unexpectedly, the reduction was also observed after a while in the unmanipulated side, which resulted in the formation of a symmetrical defocused barrel-like shape. On the other hand, it has been reported that inhibition of the dynein function by the addition of dynein-dynactin inhibitor causes the defocusing of pole regions, such that the barrel-shaped structure is formed. We compressed one side of the barrel-shaped spindle using a pair of glass micro-needles and found that the stiffness and the microtubule density in the compressed region increased. These changes also occurred in the unmanipulated side, which was accompanied by the bipolar spindle formation. Our results suggest that symmetrical shape of the spindle is dynamically balanced for proper cell division.

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Tension-Dependent Dynamic Microtubule Model for Metaphase and Anaphase Phenomena
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We present a theoretical model describing metaphase chromosome oscillations, microtubule (MT) attachment error correction, and anaphase chromosome separation. During metaphase, chromosome pairs align near the center of a bipolar MT spindle and oscillate as the MTs attaching them to the cell poles polymerize and depolymerize. Simultaneously, the cell fixes misaligned chromosome pairs by some tension-dependent mechanism. In anaphase, chromosome pairs separate as depolymerizing MTs pull each chromosome toward its respective cell pole. Instead of including all known components to develop a comprehensive, species-specific description, we introduce a minimal model based on fundamental properties of MT kinetics. We use the tension-dependence of single MT polymerization/depolymerization kinetics measured by Akiyoshi et al. [1] and assume the same functional dependence for compressed MTs. We apply these to a many MT model, and solve this stochastic model numerically and by a master equation approach. We find that the tension dependence of rates enhances the speed of single chromosome pulling by MTs during anaphase- or error-correction-like behavior. Additionally, the force-velocity curve for a single chromosome attached to dynamic MTs exhibits bi-stability: at high loads, large tension fluctuations induce MTs to spontaneously switch from a depolymerizing state into a polymerizing state. The system is hysteretic; to recover depolymerization from the polymerizing state, the load must be decreased to a far smaller value than that required to initially induce polymerization. This behavior leads to the chromosome oscillations we observe in the two-chromosome system. Interestingly, we observe breathing oscillations, which are not captured by any other chromosome oscillation model. Our minimal model reflects general features of the underlying mechanisms of these phenomena, and reveals how different components control chromosome dynamics through the rate constants.
[1] Akiyoshi et al. (2010) Nature 468, 576-579.

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Phosphoregulation of the Ndc80 and Dam1 Subcomplexes Promotes Release of Kinetochores from Dynamic Microtubules via Multiple Mechanisms
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During mitosis, multiprotein complexes called kinetochores orchestrate chromosome segregation by forming load-bearing attachments to dynamic microtubule tips, and by participating in phosphoregulatory error correction. Aurora B kinase phosphorylates the major microtubule binding subcomplexes, Ndc80 and (in yeast) Dam1, to promote release of erroneous attachments, giving another chance for proper attachments to form. It is unknown whether Aurora phosphorylation promotes release directly, by increasing the rate of kinetochore detachment, or indirectly, by destabilizing the microtubule tip. Moreover, the relative importance of phosphorylation of Ndc80 versus Dam1 in the context of whole kinetochores is unclear. To address these uncertainties, we isolated native yeast kinetochore particles carrying phosphomimetic mutations on Ndc80 and Dam1, and applied advanced laser trapping techniques to measure the strength and stability of their attachments to individual dynamic microtubule tips. Composition of the purified particles was unaffected by the phosphomimetic mutations, suggesting that phosphorylation at these sites does not disrupt kinetochore structure. Rupture forces were reduced by phosphomimetics on both subcomplexes, in an additive manner, indicating that both subcomplexes make independent contributions to attachment strength. Likewise, phosphomimetics on either subcomplex reduced attachment lifetimes under constant force, primarily by accelerating detachment during microtubule growth. Phosphomimetics on Dam1 also increased the likelihood of switches from microtubule growth into shortening, further promoting release